The PCR products produced by these primers, from a range of medically important Gram positive and Gram negative bacterial cultures, are characterized by hybridization to an array of oligonucleotides designed to identify taxonomic groups. Using this procedure, which takes typically less than four hours, we have been able to identify a wide range of genera and species. This approach allows bacteria and mixtures of bacteria to be identified by molecular methods without the need for a priori knowledge of the causative agent or agents.

In summary, the present invention comprises a method for identifying bacteria in a test sample which comprises amplifying a portion of the 23S rDNA present in the sample using a primer pair comprising one primer consisting essentially of one or more oligonucleotides having the sequence or sequences

5'GCGATTTCYGAAYGGGGRAACCC

and a second primer consisting essentially of an oligonucleotide having the sequence

5'TTCGCCTTTCCCTCACGGTACT.

and testing the resulting amplicon by probing a set of oligonucleotides designed to identify bacteria which may be present in the sample by hybridising to their respective amplicon. In a set of oligonucleotides suitable for use with this method, the oligonucleotides are designed to hybridise to the products of the amplification reaction in a single test and therefore under a single set of hybridisation conditions.

International application WO 88/09397 describes the preparation of numerous oligonucleotide probes which hybridise to certain regions of 16S and 23 S ribosomal nucleic acid. International application WO 90/14444 and US patents 5,592,978, 5,521,300 and 5,292,874 describe the preparation of individual probes which bind to certain regions of ribosomal nucleic acid but which are specific for one species of organism or one genus or sub-generic classes thereof. However, in contrast to the present invention, none of these publications disclose, either in concept or in reality, sets of oligonucleotides designed to work in unison by hybridising to a uniquely specified region of 23S ribosomal nucleic acid after amplification of bacterial nucleic acid with one specific pair of amplification primers. According to the present

invention, the sets of oligonucleotides which may be used hybridise in parallel to a range of amplicons under the same hybridisation conditions and can therefore be used in a single test for the identification of a range of different organisms.

Oligonucleotide probes, the sequences of which are set out below, have proved highly successful when used in various combinations in tests typically carried out in hospitals. They can be used in a panel or array for the identification of many different species. There is theoretically no limit to the number of oligonucleotide targets employed and the number of species that can be identified. Ideally the oligonucleotides used should each hybridize only to one bacterial species and to all members of that species. Thus with an ideal array, a unique profile consisting of species specific spots would be seen, giving identification to the species level. In practice, two or more oligonucleotide spots may be required for many species and in some cases several such spots may allow identification of variation within a species. In addition, some identifications can be made by comparing the relative intensities of hybridization of individual species to individual oligonucleotides. The assessment of hybridization can be quantified by visual or automated methods.

For example, 27 oligonucleotides have been used for the unambiguous identification of Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus feacium and Enterococcus feacalis , as well as Staphylococcus aureus, coagulase negative Staphylococcus, Listeria species, Stenotrophomonas maltophilia , Burkholderia cepacia, and Escherichia coli. Usually, therefore, it will be desirable to provide oligonucleotides to probe not only for the 8, 10, or more of the micro-organisms commonly occurring in hospital samples or the samples being tested in other situations, but also for other organisms likely to be encountered. Preferably, probes for at least 30 different species of micro-organism will be present on the support substrate used in the test.